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Origin of hypercholesterolemia in chronic experimental nephrotic syndrome

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Origin of hypercholesterolemia in chronic experimental nephrotic syndrome. Sixteen nephrotized rats and eight controls were submitted to a continuous sterol balance for two weeks. During the whole experiment (two months) the rats were pair-fed a balanced sterol-free diet and their proteinuria regularly measured as a parameter of the nephrotic state. Serum cholesterol and albumin were also measured at the end of the experiment Liver and carcass (excluding intestine and central nervous system) as well as feces were submitted to sterol analysis by gas-liquid chromatography. Sterol losses were corrected for by adding radioactive cholesterol and cholic acid at the beginning of the methodological procedures. The results showed that while fecal sterol excretion was similar in the nephrotic group as compared to controls, a definite increase in serum, carcass, and liver cholesterol was observed in the nephrotic animals, indicating that a real enhancement of synthesis had occurred. The meaning of increased cholesterol hepatic content is discussed, as well as the possible relationship between enhanced protein and cholesterol hepatic synthesis.

Origine de l'hypercholestérolémie dans le syndrome néphrotique chronique expérimental. Seize rats rendus néphrotiques et huit rats contrôles ont été soumis à une étude continue du bilan des stérols pendant deux semaines. Pendant toute la durée de l'expérience (deux mois) des paires de rats ont été alimentés avec un régime équilibré dépourvu de stérols et leur protéinurie a été régulièrement mesurée et considérée comme un paramètre de l'état néphrotique. L'albumine et le cholestérol sériques ont été mesurés à la fin de l'expérience. Le foie et le cadavre (à l'exclusion de l'intestin et du système nerveux central) de même que les selles ont été soumis à l'analyse des stérols par chromatographie gazeuse en veine liquide. Les pertes de stérols ont été corrigées par l'addition de cholestérol radioactif et d'acide cholique au début des étapes méthodologiques. Les résultats montrent qu'alors que l'excrétion fécale de stérols est semblable dans les groupes néphrotique et contrôle, une augmentation importante de la concentration de cholestérol dans le foie et le cadavre est observée chez les animaux néphrotiques, ce qui indique qu'une augmentation certaine de la synthèse est intervenue. La signification de l'augmentation du contenu hépatique en cholestérol est discutée de même que la relation possible entre les accroissements des synthèses hépatiques de protéine et de cholestérol.

In the past years several hypotheses trying to explain the hyperlipemia and the hypercholesterolemia of the nephrotic syndrome have been put forward. Some authors have related this disturbance to decreased thyroid function [1, 2, 3], but thyroxine administration does not correct elevated serum lipid levels [2]. Moreover, hypothyroidism is not a common feature in the nephrotic syndrome [4, 5]. Other authors have proposed an impaired tissue removal of lipids due to decreased lipoprotein lipase activity [6] that may be secondary to urinary loss of lipoprotein lipase [7]. Furthermore, the i.v. infusion of dextran and other macromolecules is as effective as albumin in reducing nephrotic hyperlipidemia [8], without correcting the serum albumin level. As these substances are not fatty acid carriers [8], impaired fatty acid transport secondary to low albumin values cannot be evoked to explain the primary cause of high serum lipid levels in the nephrotic syndrome [3].

On the other hand, by means of isotopic methods, evidences have been presented for lowered [9, 10], increased [10,11,12], and normal [13,14] rates of lipid synthesis. Nevertheless, no clear cut explanation can be drawn from these experiments using radiolabeled precursors such as acetate, because the size of the precursor pool was unknown or compartmental distribution was not considered.

The i.v. use of labeled products, such as radiocholesterol, was not satisfactory either since its precise rate of distribution in the tissues, a condition critical to the analysis of the data, is unknown [15]. Nevertheless, the cholesterol molecule does not enter other significant metabolic pathways, except for its transformation into bile acids, and is recovered quantitatively from animal tissues and excreted material [16, 17]. Thus, animal sterol balance studies are ideal to identify the nature of the disturbance of the serum cholesterol that occurs in the nephrotic syndrome.

In this paper the quantitative measurement of tissue cholesterol was carried out in rats made nephrotic by anti-rat kidney serum and in controls. Feces were also measured by means of the balance technique [18, 19] with the animals kept on a sterol-free diet. Therefore, by excluding participation of dietary cholesterol

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absorption, it was possible to know whether the hypercholesterolemia in nephrotic rats was due to an increased body synthesis, compartmental redistribution, or impaired fecal steroid excretion.

Methods

Male rats of Wistar origin, weighing from 90 to 100 g, were placed in individual metabolic cages and fed a sterol-free diet containing 4 calories/g during the pre-experimental week and throughout the induction of nephrosis and sterol balance period. The dietary composition by weight was 42% casein, 46% starch, 2.5% pure linoleic acid, and 9.5% mineral salts, vitamins, and methyl cellulose. The average daily intake during the whole experiment (two months) provided approximately 200 mg of linoleic acid, which is three to four times the minimum requirement [20]. The diet contained only 14μg of sterols/g as shown by gas-liquid chromatographic analysis (GLC).

Experimental nephrosis. Twenty-four rats were investigated, and sixteen were randomly chosen for the production of the experimental nephrosis (membrano-proliferative glomerulonephritis) according to the technique of Heymann and Lund [21]. A small volume of serum was injected at regular intervals to induce a mild form of the disease. Enough anti-rat kidney serum was injected to obtain a proteinuria of at least 5 mg/hr without eliciting diarrhea, ascites, or apparent edema. No deaths occurred during the experimental period. About two weeks of treatment were required to obtain the desired degree of nephrosis which was maintained throughout the ensuing experimental period. Nephrosis was controlled by daily analysis of the urinary protein excretion by the biuret method [22] and characterized at the end of the experiment by measuring serum cholesterol [23], total serum proteins [22], and serum albumin by electrophoresis on Cellogel (Chemetron, Milan, Italy).

Balance technique. After the attainment of adequate proteinuria, the rats were submitted to a 15 to 20-day continuous and complete fecal collection. As rats were fed on solid diet, no correction for sterol losses was necessary [24]. The experiment ended by exsanguinating the animals through aortic puncture during light ether anesthesia. Central nervous system and intestine were excluded from analysis since the nervous system is rich in cholesterol which behaves as an independent pool [25], and fecal contamination in the intestine is expected. The liver was separated from the remaining carcass.

Analytical procedures. Carcass, liver, and feces were analyzed separately. Tissues were digested by refluxing at 110°C on sodium hydroxide solution (aqueous for carcass, and ethanolic for liver and feces); sterols were determined by GLC [18, 19]. Methodologic procedures were tested before their use on experimental samples, and variable losses of approximately 30% were observed. Thus, cholesterol-4-^{14}C (New England Nuclear, Boston, MA) was added prior to hydrolysis to correct for neutral steroid losses, and 1,2-^{3}H cholic acid (New England Nuclear, Boston, MA) was also added to the aliquots of fecal samples to correct for bile acid losses during the analytical procedure.

Results

Considering the difficulty of controlling the weight of nephrotic rats since they might develop some degree of unnoticed edema and ascites, we resorted to pair-feeding both groups. Accordingly, the daily dietary intake was 7.9 g/rat during the whole experiment, which permitted continuous weight gain that was similar in nephrotic and control animals. Thus, the final body wt was similar in both groups; however, the carcasses were slightly heavier in control animals. Such a difference could have been due to a small amount of ascites in addition to a conceivable intestinal wall edema, besides the heavier liver in the nephrotic animals. Data on dietary intake and body wt are summarized in Figure 1.

The nephrotic state was identified by measuring

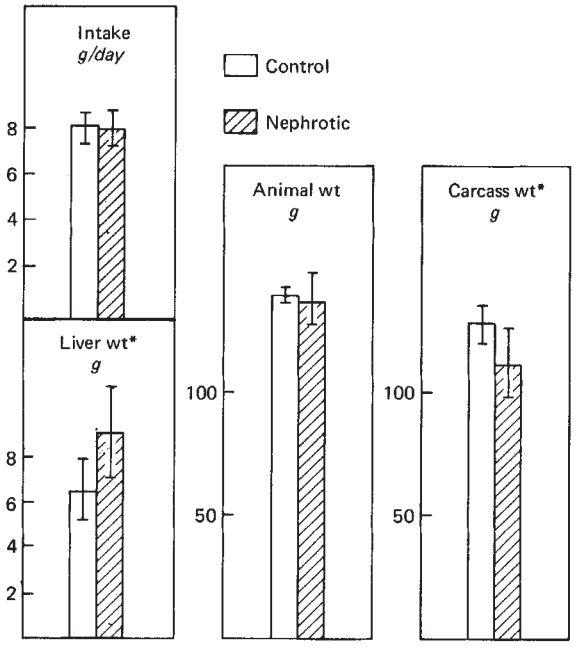

Fig. 1. *Daily dietary intake during the experiment and weights of liver, carcass, and the animal at the end of the experiment.* Values represent means ± SD of the nephrotic (N = 16) and control (N = 8) groups. The *astericks* (*) mean that the statistical difference between both groups were significant at $P < 0.01$.

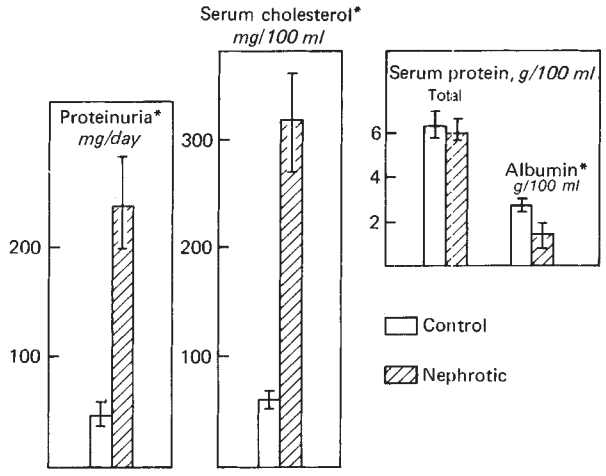

Fig. 2. *Nephrotic state of the anti-rat kidney serum injected rats, as measured by daily proteinuria, serum cholesterol, and serum albumin.* Values represent means ± SD in the nephrotic (N = 16) and control (N = 8) groups. The *astericks* (*) mean that the statistical difference between both groups were significant at $P < 0.001$.

urinary protein, serum cholesterol, and serum protein, as shown in Figure 2. Since caloric intake was identical in both groups, it is conceivable that the greater urinary loss of protein in the nephrotic rats might represent a substantial waste of calories. Considering urinary protein output in calories during the whole experiment, however, the differences between both groups amount to approximately 27 calories/month, namely only 2.5% of the total caloric intake.

Tissue cholesterol and fecal steroid balance data are presented in Table 1. Urinary sterols were excluded from calculation because they represented only 75 to 300μg/day as measured by GLC [19] and were identical in both groups. Table 1 shows a definite increase in liver and plasma cholesterol of nephrotic rats. Cholesterol level in the nephrotic carcass is higher than in controls only when expressed as mg/100 g, because carcass weight in the nephrotic group is significantly lower (Fig. 1). Even without the necessary correction for carcass weight, the analysis of whole body cholesterol shows it is increased in nephrotics as compared to controls.

Discussion

Nephrotic hypercholesterolemia may theoretically derive from: 1) increased intestinal absorption of cholesterol, 2) diminished fecal steroid output, 3) peripheral removal defect, 4) enhanced hepatic production.

In our work, animals were fed a sterol-free diet. Thus, increased intestinal absorption of cholesterol can be safely ruled out.

Fecal steroid output in nephrotics was equal to controls. As steroid excretion, however, represents a fixed fraction of cholesterol body mass [27], which is increased in the nephrotic animal, a relative impairment of steroid excretion may be present.

Previous studies dealing with cholesterol in the nephrotic syndrome suggest there is a removal defect in peripheral tissues [11] or in liver [28] as well as an enhanced body production [11, 12]. Our finding of increased tissue cholesterol mass rules out a removal impairment as the primary cause of hypercholesterolemia. In the more severe stages of the disease, however, an association with impaired peripheral removal of triglycerides due to very low serum albumin concentrations cannot be ruled out [29, 30].

Thus, enhanced hepatic production of cholesterol must be evoked to explain the increased values of cholesterol in the whole rat.

The comprehension of sterol metabolism in the liver, however, is more complex. Previous authors [31, 32] have shown that feeding cholesterol increases its content in the normal rat liver, interrupts cholesterol synthesis by a feedback mechanism, and leads to enhanced bile acid output. On the other hand, in the

Table 1. Tissue cholesterol and fecal steroid data in nephrotic and control rats[a]

	Control group (N = 8)	Nephrotic group (N = 16)
Carcass		
mg/100 g[b]	155.8 ± 10.3	183.2 ± 25.8
mg/carcass	201.0 ± 14.6	204.5 ± 30.6
Liver		
mg/100 g[b]	243.9 ± 45.0	349.6 ± 107.3
mg/liver[b]	15.6 ± 3.6	29.9 ± 7.2
Plasma		
mg/100 g[b]	64.6 ± 7.9	325.3 ± 44.8
mg/total plasma[b]	4.1 ± 0.5	20.6 ± 4.1
Whole body[c]		
mg/100 g[b]	156.4 ± 13.5	202.5 ± 23.7
mg/rat[b]	212.9 ± 16.7	259.2 ± 40.7
Neutral fecal steroids		
mg/100 g/day/BW[d]	2.7 ± 0.6	3.0 ± 0.7
mg/day	3.7 ± 0.8	3.7 ± 1.0
Fecal bile acids		
mg/100 g/day/BW[d]	4.1 ± 0.7	4.5 ± 1.5
mg/day	5.6 ± 1.0	5.4 ± 1.6

[a] Numbers represent means ± SD.
[b] Nephrotic and control groups were significantly different according to Student's t test at 1% level or higher.
[c] Whole body means the sum of carcass, liver, and plasma cholesterol. Plasma weight was calculated as 4.5% of final body weight (BW) before sacrifice [26].
[d] Body weight is expressed as the sum of carcass and liver only.

nephrotic animals, in spite of the increased hepatic content, the cholesterol feedback mechanism is operative [9], and enhanced bile acid output does not occur [33] (Table 1). These findings strongly suggest that the excess of cholesterol produced is being stored in a pool different from the production site within the hepatocyte of nephrotic rats.

The relationship between increased cholesterol synthesis and the changes in protein metabolism should also be discussed. At present, data indicate that, in experimental nephrosis, peripheral amino acids are channeled into hepatic protein synthesis [10], being simultaneously diverted from hepatic gluconeogenesis [34]. Furthermore, liver glycolysis being depressed in the nephrotic state allows a greater contingent of precursors for protein and lipid synthesis [34] and conforms to the pattern of enhanced albumin production in the liver [10, 35, 36] elicited by lowered serum oncotic pressure [35, 37]. The increased availability of the lipoprotein peptide moiety, acting as the trigger of enhanced cholesterol synthesis, becomes an interesting working hypothesis, emphasizing the connection between protein and lipid metabolism.

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